

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



B2

2

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | | |
|---|--|--|--|
| (51) International Patent Classification ⁶ : A61K 31/00 | | A2 | (11) International Publication Number: WO 00/00183 |
| | | | (43) International Publication Date: 6 January 2000 (06.01.00) |
| (21) International Application Number: PCT/IT99/00176 | | (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). | |
| (22) International Filing Date: 17 June 1999 (17.06.99) | | | |
| (30) Priority Data: RM98A000433 30 June 1998 (30.06.98) IT | | | |
| (71) Applicant (for all designated States except US): SIGMA-TAU HEALTHSCIENCE S.P.A. [IT/IT]; Via Treviso, 4, I-00040 Pomezia (IT). | | | |
| (72) Inventor; and (75) Inventor/Applicant (for US only): CAVAZZA, Claudio [IT/IT]; Piazza Campitelli, 2, I-00186 Roma (IT). | | | |
| (74) Agents: CAVATTONI, Fabio et al.; Cavattoni - Raimondi, Viale dei Parioli, 160, I-00197 Roma (IT). | | Published Without international search report and to be republished upon receipt of that report. | |
| | | | |
| (54) Title: ANTIOXIDANT, CYTOSTATIC AND SUSTAINED ENERGY COMPOSITION WHICH AMELIORATES THE METABOLIC UTILIZATION OF GLUCOSE | | | |
| (57) Abstract A composition for the prevention and/or therapeutic treatment of various alterations and pathological states induced by free radicals, that may take the form of a dietary supplement, dietetic support or of an actual medicine, is disclosed which comprises as characterizing active ingredients acetyl L-carnitine and at least one of the catechins (e.g. epigallocatechin gallate) that can be extracted from the green tea. | | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|--|----|--|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | | | TR | Turkey |
| BG | Bulgaria | HU | Hungary | ML | Mali | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MN | Mongolia | UA | Ukraine |
| BR | Brazil | IL | Israel | MR | Mauritania | UG | Uganda |
| BY | Belarus | IS | Iceland | MW | Malawi | US | United States of America |
| CA | Canada | IT | Italy | MX | Mexico | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NE | Niger | VN | Viet Nam |
| CG | Congo | KE | Kenya | NL | Netherlands | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NO | Norway | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | NZ | New Zealand | | |
| CM | Cameroon | | | PL | Poland | | |
| CN | China | KR | Republic of Korea | PT | Portugal | | |
| CU | Cuba | KZ | Kazakstan | RO | Romania | | |
| CZ | Czech Republic | LC | Saint Lucia | RU | Russian Federation | | |
| DE | Germany | LI | Liechtenstein | SD | Sudan | | |
| DK | Denmark | LK | Sri Lanka | SE | Sweden | | |
| EE | Estonia | LR | Liberia | SG | Singapore | | |

Antioxidant, cytostatic and sustained energy composition which ameliorates the metabolic utilization of glucose.

The present invention relates to a composition for:

- (a) preventing and/or treating diseases brought about by the presence of free radicals due to environmental pollution; brain or myocardial damages induced by free radicals following cerebral or myocardial ischaemia and attendant reperfusion; atherosclerotic damages and tissue proliferative processes; cataract and diabetic neuropathy;
- (b) increasing glucose utilization in diabetics or subjects with an insufficient response to insulin activity; and
- (c) promoting muscle adaptation to programs of strenuous exercise.

Accordingly, the composition may take the form and exert the action of a dietary supplement or of an actual medicine, depending upon the support or preventive action, or the strictly therapeutic action, which the composition is intended to exert in relation to the particular individuals it is to be used in.

More particularly, the present invention relates to an orally, parenterally, rectally or transdermally administrable composition which comprises in combination:

- (a) acetyl L-carnitine or a pharmacologically acceptable salt thereof, optionally in combination with at least a further "carnitine" where for "carnitine" is intended L-carnitine or an alkanoyl L-carnitine selected from the group comprising propionyl-L-carnitine, valeryl L-carnitine, isovaleryl L-carnitine or their pharmacologically acceptable salts; and
- (b) a catechin selected from the group comprising epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate or mixture thereof.

It is well known that L-carnitine and its derivatives have proved useful in the treatment of ischaemic heart disease, angina pectoris, peripheral vascular disease, situations of increased muscular energy requirement and the various forms of atherosclerosis.

These therapeutic activities of carnitine and its derivatives are related to the complex biochemical activity which this substance is capable of exerting at cell and tissue level.

In addition to being essential for the β -oxidation of fatty acids and activating ATP synthesis, carnitine plays an important role as an antioxidant, as demonstrated by its protective effect against hyperoxidation of the cell phospholipid membranes and against the oxidative stress induced at myocardial and endothelial cell level. In particular, carnitine has also proved capable of intervening in carbohydrate metabolism and insulin secretion. In performing these various activities, all the various carnitines studied prove effective, though with a different intensity of action, with the result that, in many cases, it would appear to be more useful to administer the various carnitines in combination rather than any single carnitine alone. This is probably due to their different kinetics and different types of intervention in the stages of mitochondrial functional activation, which results in an amplification of the metabolic effect desired. The carnitines indicated as being interactive with one another are mostly L-carnitine, acetyl L-carnitine, propionyl L-carnitine and isovaleryl L-carnitine.

It has also been demonstrated that the polyphenols, too, and, in particular, the catechins present in green tea perform an important regulatory function on carbohydrate metabolism and insulin activity, as well as on tissue proliferative reactions and oxidising activity.

Though the beneficial effects of tea on human health have long been known, it has only recently proved possible to isolate the most important soluble tea components so as to be able to study their pharmacological properties.

Using chromatographic fractionation methods, it has proved possible to isolate at least five different catechins from green tea as active components, namely: (-)gallo-catechin (GC), (-)epigallocatechin (EGC),

(-)-epicatechin (EC), (-)-epigallocatechin gallate (EGCg) and (-)-epicatechin gallate (ECg).

In the case of black tea, whether of tropical origin or obtained by various extraction processes, the catechins are oxidised and preferably form theaflavins and thearubigins, which are also endowed, albeit to a lesser extent, with pharmacological activity. Epidemiological studies in populations submitted to the same degree of oxidative stress, such as that due to environmental pollutants or to cigarette smoking, in equally industrially developed countries of the Far East, have demonstrated a lower incidence of cardiovascular accidents in populations such as the Japanese and Chinese, where, unlike others, there is extensive consumption of tea as a beverage, so much so, indeed, as to suggest a "Far East Paradox" along the lines of the "French Paradox".

The antioxidant capacity of the total catechins present in green tea or of the crude catechins, as well as of the various catechins considered singly, has been evaluated in numerous experimental and clinical tests. An intense antioxidant capacity has been demonstrated for all the various different catechins, for example on linoleic acid and on saturated fatty acids such as lard; this capacity is very marked even as compared with other antioxidants such as, for instance, α -tocopherol.

Significant results have also been obtained in experiments conducted on the low-density lipoprotein (LDL) oxidation induced by copper salts, which was inhibited both by total crude catechins and by epigallocatechin gallate.

The ability of tea catechins to inhibit LDL oxidation mediated by the presence of macrophages has also been demonstrated in an *in-vivo* study.

In addition to the level of lipid antioxidant capacity, one of the biological effects of the tea catechins most extensively studied is the regulation of carbohydrate metabolism.

The administration of tea catechins is, in fact, capable of reducing the serum glucose and insulin levels induced by the administration of starch or sucrose in the rat. In animals with streptozotocin-induced diabetes, it has also been shown that the administration of tea catechins reduces serum glucose and may exert a preventive and curative effect on the occurrence of diabetes.

In addition to their antioxidant activities, then, it has also been demonstrated that tea catechins are capable of intervening in carbohydrate metabolism and insulin secretion, as well as in regulating tissue proliferative processes.

The risk of pancreatic tumours and oncogene expression can be reduced by administration of tea.

Research studies conducted by subcutaneously injecting a carcinogen such as methylcholantrene in the mouse have demonstrated that tea catechins can delay the onset of subcutaneous solid tumours in these animals as well as the onset of spontaneous mammary tumours in female mice (C3H/HeN stock).

These beneficial effects of the catechins present in green tea may be due to stimulation of glutathione peroxidase and catalase, and to inhibition of ornithine decarboxylase and urokinase.

Among the various catechins studied, epigallocatechin gallate is the one which has proved most active in inhibiting tumour necrosis factor (TNF) and ornithine decarboxylase mRNA gene expression.

Surprisingly, it has been found that a composition comprising, as its characterising components, a combination of:

(a) acetyl L-carnitine or a pharmacologically acceptable salt thereof; and

(b) a catechin selected from the group consisting of (-)epicatechin, (-)epicatechin gallate, (-)epigallocatechin and (-)epigallocatechin gallate, or mixtures thereof,

is extremely effective in the prevention and therapeutic treatment of damage induced by the presence of free radicals due to environmental pollution, cerebral or myocardial lesions induced by free radical after cerebral or myocardial ischaemia and as a result of reperfusion; in the prevention and treatment of atherosclerotic lesions and tissue proliferative processes and cataract; to increase the glucose utilisation capacity in diabetic subjects or in subjects with an inadequate insulin activity response; in the prevention and treatment of diabetic neuropathies and in situations of increased muscular energy requirement, owing to the potent synergistic effect of its components.

It has also been found that, advantageously, component (a) can further comprise a "carnitine" selected from the group consisting of L-carnitine, propionyl L-carnitine, valeryl L-carnitine, isovaleryl L-carnitine or their pharmacologically acceptable salts, or mixtures thereof, and that component (b) may consist of a green tea extract (*Camellia sinensis*, *Camellia thea* Link, *Theaceae* family).

The (a):(b) weight-to-weight ratio ranges from 1:0.1 to 1:1.

Toxicology

The low toxicity and good tolerability of both tea catechins and carnitines are well known. These favourable toxicological characteristics of catechins and carnitines have been confirmed by combining these components and administering them at high doses to both rats and mice. In these animals, it proved possible, in fact, to administer parenterally more than 75 mg/kg of catechin complex and 200 mg/kg of carnitines (50 mg L-carnitine + 50 mg/kg acetyl L-carnitine + 50 mg propionyl L-carnitine + 50 mg isovaleryl L-carnitine) or 250 mg/kg of

acetyl L-carnitine, and more than 500 mg/kg catechin complex and 400 mg/kg of carnitines (combined in identical weight amount to one another), or 400 mg/kg of acetyl L-carnitine orally, without any of the animals thus treated dying. Prolonged administration with the diet for thirty days consecutively, in both a group of rats and a group of mice, of a combination of 200 mg/kg of catechin complex or 50 mg/kg of epigallocatechin plus 200 mg/kg of carnitine mixture or 250 mg/kg of acetyl L-carnitine was also well tolerated and led to the detection of no signs of toxicity. Both the weight increase and the various blood chemistry examinations performed in these animals showed normal values, as did the histopathological examinations performed on the main organs after sacrificing the animals at the end of treatment.

Tests of neuroprotective activity in experimental cerebral ischaemia

In view of the fact that the lesions caused by cerebral ischaemia are related to the production of free radicals and nitrous oxide (Lipton, S.A., Nature 364, 625, 1993) and in view of the favourable protective effect of both carnitines and green-tea catechins against the damage caused by free radicals, it was decided to evaluate their activity on experimental cerebral ischaemia. To this end, cerebral ischaemia was induced by occluding the middle cerebral artery (MCA) according to the method described by Scharkey (Scharkey, Y., Nature, 371-336, 1994) by 3-minute injections of endothelin-1 (120 pmol in 3 nl) in anaesthetised rats via a microcannula positioned stereotactically in the piriform cortex at the level of the middle cerebral artery. Occlusion of the artery is induced, and the resulting ischaemic area can be checked three days after this procedure by transcardiac perfusion of a solution of paraformaldehyde (4% in PBS).

After removing the brain, the latter was placed for 24 hours in a fixative containing 10% sucrose, and the cryostatic sections (20 nm) were fixed with cresyl violet and examined under the optical microscope. Carnitine mixture (50 mg/kg of a combination consisting of L-carnitine + acetyl L-carnitine + propionyl L-carnitine + isovaleryl L-

carnitine in identical weight amount to one another, acetyl L-carnitine (50 mg/kg), green-tea catechin complex (50 mg/kg), epigallocatechin gallate (50 mg/kg), or various combinations of these products, were administered intravenously 5 minutes after injection of endothelin.

The volume of the infarcted area was calculated according to the method described by Park (Park, C.K., Anns. Neurol., 20, 150, 1989). The results of these tests (Table 1) demonstrate that the carnitine mixture, acetyl L-carnitine, the catechin complex and epigallocatechin gallate are equally capable of reducing the ischaemic area, but a surprisingly greater and more significant result is achieved only with the combination of the carnitine mixture plus the catechin complex or with epigallocatechin gallate plus acetyl L-carnitine.

An unforeseeable synergistic action produced by the combination of these products was thus demonstrated.

Table 1

Magnitude of ischaemia (volume in mm³) induced by occlusion of the MCA (percentage reduction in volume compared to controls).

| | Volume (mm ³) |
|---|---------------------------|
| Carnitine mixture | 25.6 ± 1.9 |
| Acetyl L-carnitine | 27.2 ± 2.2 |
| Catechin complex | 33.5 ± 2.5 |
| Epigallocatechin gallate | 30.7 ± 3.3 |
| Carnitine mixture + catechin complex | 86.7 ± 4.7 |
| Carnitine mixture + epigallocatechin gallate | 80.2 ± 5.1 |
| Acetyl L-carnitine + catechin complex | 80.5 ± 4.9 |
| Acetyl L-carnitine + epigallocatechin gallate | 89.7 ± 5.3 |

Test of reduction of lens opacification in galactosaemic rats induced by the administration of carnitine mixture, acetyl L-carnitine, catechin complex, epigallocatechin gallate, or combinations of these products

The administration of galactose with the diet causes the onset of ocular cataracts in rats. The opacification of the lens which occurs after

approximately eight days of treatment is classified in increasing order of severity as stages I, II or III, according to the method described by Sippel (Sippel T.O., Invest. Ophthalmol., 5, 568, 1966). Treatment for eight days, together with the galactose-containing diet, with carnitine mixture (300 mg/kg of a combination of L-carnitine + acetyl L-carnitine + propionyl L-carnitine + isovaleryl L-carnitine in an identical weight amount to one another), acetyl L-carnitine (300 mg/kg), catechin complex (100 mg/kg), or epigallocatechin gallate (50 mg/kg), or with various combinations of these products, induces a reduction in the severity of lens opacification. The combination of carnitine mixture plus catechin complex, or, better still, the combination of acetyl L-carnitine plus epigallocatechin gallate almost completely inhibits the onset of opacification (Table 2). These tests, then, also demonstrate an intense, unexpected synergistic effect produced by the combination of the various components.

Table 2

Tests of inhibition of lens opacification in galactosaemic rats treated with carnitine mixture, acetyl L-carnitine, catechin complex or epigallocatechin gallate or with various combinations of these products (groups of 10 rats).

| Treatment (mg/kg) | Degree of lens opacification (number of lenses examined) | | |
|---|---|----|-----|
| | I | II | III |
| Controls | 0 | 10 | 10 |
| Carnitine mixture | 5 | 16 | 4 |
| Acetyl L-carnitine | 5 | 15 | 5 |
| Catechin complex | 8 | 8 | 4 |
| Epigallocatechin gallate | 9 | 7 | 4 |
| Carnitine mixture + catechin complex | 16 | 4 | 0 |
| Carnitine mixture + epigallocatechin gallate | 15 | 5 | 0 |
| Acetyl L-carnitine + epigallocatechin gallate | 16 | 4 | 0 |

Tests of reduction of sorbitol content in the lens and sciatic nerve in rats with streptozotocin-induced diabetes

In view of their ability to intervene in carbohydrate metabolism, it was decided to test whether the administration of the carnitine mixture, or acetyl L-carnitine, or the catechin complex or epigallocatechin gallate, or of various combinations of these substances could improve insulin control of high glucose concentrations and prevent the occurrence of the most frequent diabetic complications such as neuropathies, retinopathies, or cataracts.

High glucose concentrations not controlled by insulin can lead, as is well known, to intracellular accumulation of sorbitol, with a reduction in cell integrity and osmotic capacity. In these tests, groups of rats with streptozotocin-induced diabetes received oral administrations of carnitine mixture (300 mg/kg of a combination of L-carnitine + acetyl L-carnitine + propionyl L-carnitine + isovaleryl L-carnitine in an identical weight amount to one another), or catechin complex (100 mg/kg), or epigallocatechin gallate (50 mg/kg), or various combinations of these products at the same doses. After eight days of treatment and suitable chemical isolation, the sorbitol concentration present in the lens and sciatic nerve of the animals thus treated was measured.

A reduction in sorbitol concentration was observed in the animals treated with carnitine mixture, acetyl L-carnitine, catechin complex, or epigallocatechin gallate, but was distinctly greater in the animals treated with the combinations, thus confirming the potent synergistic effect of their components (Table 3).

Table 3

Reduction of sorbitol concentrations in the lens and sciatic nerve induced by the administration of carnitine mixture, acetyl L-carnitine, catechin complex, or epigallocatechin gallate, or various combinations of these, in rats with streptozotocin-induced diabetes.

| Treatment | Sorbitol (nmol/mg) | |
|---|--------------------|------------------|
| | Lens | Sciatic nerve |
| Controls | 0.55 ± 0.04 | 0.085 ± 0.01 |
| Diabetic rats | 38.25 ± 3.1 | 1.405 ± 0.11 |
| Carnitine mixture | 34.70 ± 2.9 | 1.260 ± 0.14 |
| Acetyl L-carnitine | 32.75 ± 2.6 | 1.125 ± 0.10 |
| Catechin complex | 25.25 ± 3.7 | 0.830 ± 0.22 |
| Epigallocatechin gallate | 27.55 ± 3.3 | 0.705 ± 0.15 |
| Carnitine mixture + catechin complex | 19.40 ± 2.75 | 0.405 ± 0.10 |
| Carnitine mixture + epigallocatechin gallate | 16.80 ± 2.55 | 0.395 ± 0.00 |
| Acetyl L-carnitine + epigallocatechin gallate | 14.75 ± 3.1 | 0.290 ± 0.01 |

Antiproliferation tests and evaluation of ornithine decarboxylase

The method used to assess antiproliferative activity consisted in the sub-cutaneous injection in mice of teleocidin, which, like the phorbol-myristates, causes proliferative abnormalities at the level of the animal's skin which can lead to the formation of cancer-type keratotic processes (Fujiki, H., Biochem. Biophys. Res. Comm., 90, 976, 1979).

The cutaneous abnormalities are accompanied by an increase in the enzyme ornithine decarboxylase and this increase is proportional to the severity of the lesion induced, and can therefore be used as a marker of the tumour proliferation reaction.

Teleocidin was injected subcutaneously in the depilated backs of the mice at a dose of 5 mcg/mouse dissolved in 0.2 cc of aqueous solution. Carnitine mixture (300 mg/kg of a combination of L-carnitine + acetyl L-carnitine + propionyl L-carnitine + isovaleryl L-carnitine in an identical weight amount to one another), or acetyl L-carnitine alone (300 mg/kg), or catechin complex extracted from green tea (100 mg/kg), or epigallocatechin gallate (50 mg/kg), or various combinations of these were administered orally to the animals for the seven-day period

preceding the test, or were applied to the skin area half an hour before injection of teleocidin after suitable dispersion in lanolin so as to reach a 200 mg/cc concentration of carnitine mixture or acetyl L-carnitine, or a 100 mg/cc concentration of catechin complex, or a 50 mg/cc concentration of epigallocatechin gallate.

0.33 cc of these preparations were applied to the skin area where the teleocidin was injected and the treated area was protected with occlusive bandages.

The ornithine decarboxylase assay was performed on the homogenised epidermis of both the orally and locally treated animals five hours after injection of teleocidin according to the method described by O'Brien and Nakadata (O'Brien, T.G., Cancer Res., 42, 2841, 1982).

The protein concentration of the epidermal extract was measured according to the method described by Lowry (Lowry, O.H., J. Biol. Chem., 193, 265, 1951).

The results obtained in these tests (Table 4) demonstrate that the modest protection against cutaneous proliferative phenomena and increased ornithine decarboxylase activity afforded by the carnitine mixture, or by the catechin complex, or by epigallocatechin gallate is surprisingly much greater and more easily detectable when the carnitine mixture is combined with the catechin complex or with epigallocatechin gallate.

The results of these tests, too, then, demonstrate the surprising, unexpected synergistic effect of the various components of the compositions according to the invention.

Table 4

Ornithine decarboxylase activity

| Treatment | Ornithine decarboxylase activity (nMol of CO ₂ /60 min/mg protein) | |
|---|--|--------------|
| Controls | 0.06 ± 0.007 | 0.04 ± 0.004 |
| Teleocidin | 2.3 ± 0.3 | 2.7 ± 0.2 |
| Carnitine mixture | 1.96 ± 0.4 | 1.85 ± 0.03 |
| Acetyl L-carnitine | 1.85 ± 0.5 | 1.70 ± 0.04 |
| Catechin complex | 1.88 ± 0.2 | 1.80 ± 0.03 |
| Epigallocatechin gallate | 1.75 ± 0.4 | 1.68 ± 0.05 |
| Carnitine mixture + catechin complex | 0.45 ± 0.07 | 0.65 ± 0.08 |
| Carnitine mixture + epigallocatechin gallate | 0.40 ± 0.05 | 0.45 ± 0.06 |
| Acetyl L-carnitine + epigallocatechin gallate | 0.37 ± 0.06 | 0.30 ± 0.07 |

Some illustrative, non-limiting examples of formulations according to the invention are reported hereinbelow.

- 1) Carnitine mixture mg 500
(L-carnitine mg 125, acetyl L-carnitine mg 125, propionyl L-carnitine mg 125, isovaleryl L-carnitine mg 125)

Catechin complex mg 200
(green tea extract with around 60% of polyphenols concentration - of which 40% is epigallocatechin gallate)
- 2) Carnitine mixture mg 500
(L-carnitine mg 125, acetyl L-carnitine mg 125, propionyl L-carnitine mg 125, isovaleryl L-carnitine mg 125)

Catechin complex mg 200
(green tea extract with around 90% of polyphenols concentration - of which 50% is epigallocatechin gallate)
- 3) Carnitine mixture mg 300
(L-carnitine mg 75, acetyl L-carnitine mg 75, propionyl L-carnitine mg 75, isovaleryl L-carnitine mg 75)

Catechin complex mg 100
(green tea extract with around 60% of polyphenols concentration - of which 40% is epigallocatechin gallate)

- | | | | |
|-----|---|----|-----|
| 4) | Carnitine mixture (L-carnitine mg 75, acetyl L-carnitine mg 75, propionyl L-carnitine mg 75, isovaleryl L-carnitine mg 75) | mg | 300 |
| | Catechin complex (green tea extract with around 90% of polyphenols concentration - of which 50% is epigallocatechin gallate) | mg | 100 |
| 5) | Carnitine mixture (L-carnitine mg 125, acetyl L-carnitine mg 125, propionyl L-carnitine mg 125, isovaleryl L-carnitine mg 125) | mg | 500 |
| | Epigallocatechin gallate | mg | 200 |
| 6) | Carnitine mixture (L-carnitine mg 75, acetyl L-carnitine mg 75, propionyl L-carnitine mg 75, isovaleryl L-carnitine mg 75) | mg | 300 |
| | Epigallocatechin gallate | mg | 100 |
| 7) | Acetyl L-carnitine | mg | 500 |
| | Catechin complex (green tea extract with around 60% of polyphenols concentration - of which 40% is epigallocatechin gallate) | mg | 200 |
| 8) | Acetyl L-carnitine | mg | 500 |
| | Catechin complex (green tea extract with around 90% of polyphenols concentration - of which 50% is epigallocatechin gallate) | mg | 200 |
| 9) | Acetyl L-carnitine | mg | 300 |
| | Catechin complex (green tea extract with around 60% of polyphenols concentration - of which 40% is epigallocatechin gallate) | mg | 100 |
| 10) | Acetyl L-carnitine | mg | 300 |
| | Catechin complex (green tea extract with around 90% of polyphenols concentration - of which 50% is epigallocatechin gallate) | mg | 100 |
| 11) | Acetyl L-carnitine | mg | 500 |
| | Epigallocatechin gallate | mg | 200 |

| | | | |
|-----|---|------|-----|
| 12) | Acetyl L-carnitine | mg | 300 |
| | Epigallocatechin gallate | mg | 100 |
| 13) | Carnitine mixture (L-carnitine mg 75, acetyl L-carnitine mg 75, propionyl L-carnitine mg 75, isovaleryl L-carnitine mg 75) | mg | 300 |
| | Catechin complex (green tea extract with around 90% of polyphenols concentration - of which 50% is epigallocatechin gallate) | mg | 100 |
| | β -carotene | mg | 10 |
| | CoQ10 | mg | 10 |
| | Vit. E | mg | 10 |
| | Magnesium stearate | mg | 10 |
| | Zinc glycinate | mg | 10 |
| | Selenium methionine | mg | 0.2 |
| 14) | Acetyl L-carnitine | mg | 300 |
| | Epigallocatechin gallate | mg | 100 |
| | CoQ10 | mg | 10 |
| | Vit. E | mg | 10 |
| | β -carotene | mg | 10 |
| | Vit. C | mg | 50 |
| | Vit. B1 | mg | 2 |
| | Vit. B2 | mg | 2 |
| | Vit. B6 | mg | 1 |
| | Vit. B12 | mcg | 50 |
| | Folic acid | mcg | 100 |
| | Vit. PP | mg | 20 |
| | Vit. D | U.I. | 500 |
| | Calcium pantothenate | mg | 15 |
| | Magnesium stearate | mg | 5 |
| | Zinc glycinate | mg | 10 |
| | Selenium methionine | mg | 0.2 |

What is meant by pharmacologically acceptable salt of L-carnitine or alkanoyl L-carnitine is any salt of these active ingredients with an acid that does not give rise to unwanted toxic or side effects. These acids are well known to pharmacy experts.

Non-limiting examples of suitable salts are the following: chloride; bromide; iodide; aspartate, acid aspartate; citrate, acid citrate; tartrate; phosphate, acid phosphate; fumarate; acid fumarate; glycerophosphate; glucose phosphate; lactate; maleate, acid maleate; orotate; oxalate, acid oxalate; sulphate, acid sulphate, trichloroacetate, trifluoroacetate and methane sulphonate.

A list of FDA-approved pharmacologically acceptable salts is given in Int. J. of Pharm. 33, (1986), 201-217; this latter publication is incorporated herein by reference.

The composition according to the invention may also comprise vitamins, coenzymes, minerals substances and antioxidants.

Appropriate excipients to be used to prepare the compositions having regards to the specific route of administration, will be apparent to the pharmacy and food industry experts.

Claims

1. A composition which comprises:
 - (a) acetyl L-carnitine or a pharmacologically acceptable salt thereof; and
 - (b) a catechin selected from the group comprising epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate or mixture thereof.
2. The composition of claim 1, wherein the ingredient (a) further comprises a "carnitine" selected from the group comprising L-carnitine, propionyl L-carnitine, valeryl L-carnitine, isovaleryl L-carnitine or their pharmacologically acceptable salts or mixtures thereof.
3. The composition of claim 1 or 2 wherein the weight ratio (a):(b) is from 1:0.01 to 1:1.
4. The composition of any of the preceding claims, wherein the ingredient (b) is in the form of vegetal extracts which contain the ingredient itself.
5. The composition of claim 4, wherein said vegetal extract is a green tea extract.
6. The composition of any of the preceding claims wherein the pharmacologically acceptable salt of L-carnitine or alkanoyl L-carnitine is selected from the group comprising: chloride; bromide; iodide; aspartate, acid aspartate; citrate, acid citrate; tartrate; phosphate, acid phosphate; fumarate, acid fumarate; glycerophosphate; glucose phosphate; lactate; maleate, acid maleate; orotate; acid oxalate; sulphate, acid sulphate; trichloroacetate; trifluoroacetate and methane sulphonate.
7. The composition of any of the preceding claims, which further comprises vitamins, coenzymes, mineral substances and antioxidants.

8. The composition of any of the preceding claims, orally administrable, in the form of a dietary supplement.
9. The composition of any of the preceding claims, orally, parenterally, rectally or transdermally administrable in the form of a medicament.
10. The dietary supplement of claim 8, for:
 - (a) preventing diseases brought about by the presence of free radicals due to environmental pollution; brain or myocardial damages induced by free radicals following cerebral or myocardial ischaemia and attendant reperfusion; atherosclerotic damages and tissue proliferative processes; cataract and diabetic neuropathy;
 - (b) increasing glucose utilization in diabetics or subjects with an insufficient response to insulin activity; and
 - (c) promoting muscle adaptation to programs of strenuous exercise.
11. The medicament of claim 9, for treating diseases brought about by the presence of free radicals due to environmental pollution; brain or myocardial damages induced by free radicals following cerebral or myocardial ischaemia and attendant reperfusion; atherosclerotic damages and tissue proliferative processes; cataract and diabetic neuropathy.
12. The dietary supplement of claim 8 or 10, in solid, semi-solid or liquid form.
13. The medicament of claim 9 or 11, in solid, semi-solid or liquid form.
14. The dietary supplement of claim 12, in the form of pills, tablets, capsules, granulates or syrup.
15. The medicament of claim 13, in the form of pills, tablets, capsules, granulates, syrups, vials, drops and collyria.



PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | | |
|---|-----------|---|--|
| (51) International Patent Classification ⁷ : A61K 35/78, 39/06, A61P 9/10, 25/02, 27/12, A61K 31/35 // (A61K 31/35, 31:22) (A61K 35/78, 31:22) | A3 | (11) International Publication Number: WO 00/00183 | (43) International Publication Date: 6 January 2000 (06.01.00) |
|---|-----------|---|--|

(21) International Application Number: PCT/IT99/00176

(22) International Filing Date: 17 June 1999 (17.06.99)

(30) Priority Data:
RM98A000433 30 June 1998 (30.06.98) IT(71) Applicant (for all designated States except US): SIGMA-TAU
HEALTHSCIENCE S.P.A. [IT/IT]; Via Treviso, 4, I-00040
Pomezia (IT).

(72) Inventor; and

(75) Inventor/Applicant (for US only): CAVAZZA, Claudio
[IT/IT]; Piazza Campitelli, 2, I-00186 Roma (IT).(74) Agents: CAVATTONI, Fabio et al.; Cavattoni - Raimondi,
Viale dei Parioli, 160, I-00197 Roma (IT).(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG,
BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB,
GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG,
KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK,
MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI,
SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA,
ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ,
UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD,
RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK,
ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI
patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR,
NE, SN, TD, TG).

Published

With international search report.

(88) Date of publication of the international search report:
29 June 2000 (29.06.00)(54) Title: ANTIOXIDANT, CYTOSTATIC AND SUSTAINED ENERGY COMPOSITION WHICH AMELIORATES THE
METABOLIC UTILIZATION OF GLUCOSE COMPRISING ACETYL-L-CARNITINE AND A CATECHIN

(57) Abstract

A composition for the prevention and/or therapeutic treatment of various alterations and pathological states induced by free radicals, that may take the form of a dietary supplement, dietetic support or of an actual medicine, is disclosed which comprises as characterizing active ingredients acetyl L-carnitine and at least one of the catechins (e.g. epigallocatechin gallate) that can be extracted from the green tea.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|--|----|--|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | ML | Mali | TR | Turkey |
| BG | Bulgaria | HU | Hungary | MN | Mongolia | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MR | Mauritania | UA | Ukraine |
| BR | Brazil | IL | Israel | MW | Malawi | UG | Uganda |
| BY | Belarus | IS | Iceland | MX | Mexico | US | United States of America |
| CA | Canada | IT | Italy | NE | Niger | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NL | Netherlands | VN | Viet Nam |
| CG | Congo | KE | Kenya | NO | Norway | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NZ | New Zealand | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | PL | Poland | | |
| CM | Cameroon | KR | Republic of Korea | PT | Portugal | | |
| CN | China | KZ | Kazakstan | RO | Romania | | |
| CU | Cuba | LC | Saint Lucia | RU | Russian Federation | | |
| CZ | Czech Republic | LI | Liechtenstein | SD | Sudan | | |
| DE | Germany | LK | Sri Lanka | SE | Sweden | | |
| DK | Denmark | LR | Liberia | SG | Singapore | | |
| EE | Estonia | | | | | | |

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/IT 99/00176

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K35/78 A61K39/06 A61P9/10 A61P25/02 A61P27/12
A61K31/35 //(A61K31/35,31:22), (A61K35/78,31:22)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| P, X | WO 98 33494 A (KOSBAB JOHN V) 6 August 1998 (1998-08-06) page 34, line 11-19; claims 1,7,16,20,27-31; example 1; tables 2-4 page 27, line 7 -page 28, line 5 page 18, line 27-30 page 26, line 27 -page 27, line 2 page 25, line 6-27 | 1-15 |
| X | US 5 667 791 A (WARSHAW MICHAEL A ET AL) 16 September 1997 (1997-09-16) column 9, line 19-30; claims 3,4,13-15; example 3 column 3, line 43-62; examples 1,2,4,5 | 1,4-7, 12-15 |
| X | WO 97 02041 A (CRANDALL WILSON T) 23 January 1997 (1997-01-23) the whole document | 1,6,7,9, 12-15 |
| | -/- | |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

11 February 2000

Date of mailing of the international search report

24/02/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3018

Authorized officer

Kanbier, D

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/IT 99/00176

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| A | EP 0 773 020 A (SIGMA TAU IND FARMACEUTI) 14 May 1997 (1997-05-14) claims 1-4,6-10 | 1,2,4,6, 7,9-15 |
| A | SCHOEMAKER: "Pharmacological Treatment of Diabetic Peripheral Neuropathy: Challenges and Possibilities" BRITISH JOURNAL OF CLINICAL PRACTICE , vol. 48, no. 2, 1994, pages 91-96, XP000878610 page 94, left-hand column; table 1 page 95, right-hand column, paragraph 3 | 1,2,4, 7-11 |
| A | FR 2 388 556 A (SIGMA TAU IND FARMACEUTI) 24 November 1978 (1978-11-24) claims 1,2 | 1,2,6-15 |
| A | SOLIMAN ET AL: "In Vitro Attenuation of Nitric Oxide Production in C6 Astrocyte Cell Culture by Various Dietary Compounds" PROC. SOC. EXP. BIOL. MED., vol. 218, no. 4, 1998, pages 390-397, XP000867853 page 390, left-hand column page 392 | 1,4,5, 8-11 |
| A | MATSUOKA ET AL: "Ameliorative Effects of Tea Catechins on Active Oxygen-Related Nerve Cell Injuries" JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, vol. 274, no. 2, 1995, pages 602-608, XP000878596 the whole document | 1,4,5, 8-11 |
| A | HOTTA ET AL: "Effects of Propionyl-L-Carnitine and Insulin on the Electroretinogram, Nerve Conduction and Nerve Blood Flow in Rats with Streptozotocin-Induced Diabetes" PFLUEGERS ARCHIV EUROPEAN JOURNAL OF PHYSIOLOGY, vol. 431, no. 4, 1996, pages 564-570, XP000874774 page 569, left-hand column | 1,2,9-11 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IT 99/00176

| Patent document cited in search report | | Publication date | Patent family member(s) | Publication date |
|---|---|---------------------|----------------------------|---------------------|
| WO 9833494 | A | 06-08-1998 | AU 6141498 A | 25-08-1998 |
| US 5667791 | A | 16-09-1997 | US 5840681 A | 24-11-1998 |
| WO 9702041 | A | 23-01-1997 | AU 6482596 A | 05-02-1997 |
| EP 0773020 | A | 14-05-1997 | IT RM950687 A | 17-04-1997 |
| | | | CA 2187990 A | 18-04-1997 |
| | | | JP 9165331 A | 24-06-1997 |
| | | | US 5747536 A | 05-05-1998 |
| FR 2388556 | A | 24-11-1978 | IT 1155813 B | 28-01-1987 |
| | | | IT 1126725 B | 21-05-1986 |
| | | | AU 3459878 A | 04-10-1979 |
| | | | BE 866568 A | 14-08-1978 |
| | | | CH 636268 A | 31-05-1983 |
| | | | DE 2817358 A | 02-11-1978 |
| | | | GB 1596491 A | 26-08-1981 |
| | | | IE 46703 B | 24-08-1983 |
| | | | JP 1015483 B | 17-03-1989 |
| | | | JP 1531621 C | 24-11-1989 |
| | | | JP 53136523 A | 29-11-1978 |
| | | | KE 3223 A | 03-09-1982 |
| | | | NL 7804567 A,B, | 31-10-1978 |
| | | | US 4194006 A | 18-03-1980 |
| | | | AU 518617 B | 08-10-1981 |
| | | | ZA 7801981 A | 28-03-1979 |

THIS PAGE BLANK (USPTO)